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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/435,403	11/05/1999	JOHN S. LOLLAR	88-98	5191

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EXAMINER	
SCHNIZER, HOLLY G	
ART UNIT	**PAPER NUMBER**
1653	

DATE MAILED: 11/05/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/435,403	LOLLAR, JOHN S.	
	Examiner	Art Unit	
	Holly Schnizer	1653	

-- The MAILING DATE f this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 July 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5 and 6* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *6* is/are allowed.

6)☒ Claim(s) *1 and 5* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)	4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) ☐ Notice of Informal Patent Application (PTO-152)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .	6) ☐ Other: .

DETAILED ACTION

Status of the Claims

The Amendment filed July 24, 2003 has been entered and considered. Clams 2-4 have been cancelled and Claim 6 has been added. Therefore, Claims 1, 5, and 6 are pending and have been considered in this Office Action.

Rejections Maintained

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1 and 5 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for preparing a factor VIII molecule having modified glycosylation wherein a specific mutation disclosed in the Specification is made, does not reasonably provide enablement for a method for preparing a factor VIII protein having modified glycosylation comprising making a mutation anywhere in the protein sequence, *or anywhere in the A2 or C2 domains to insert a glycosylation site*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

3. The factors to be considered in determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art and, (8) the breadth of the claims. In the present case, it appears that undue experimentation would be required to practice the claimed method to successfully produce a functional factor VIII protein having the structural limitations of the claims.

4. Applicants argue that the experimentation required to practice the invention is routine and not undue because protocols for site directed mutagenesis, protein expression, and testing for biological activity are well known and readily available. This argument has been considered but is not deemed persuasive because this is not adequate guidance as to the nature of the factor VIII mutants that that may be successfully prepared using the claimed method, but is merely an invitation to the artisan to further experiment to find sites in factor VIII wherein introduction of glycosylation sites would not disturb its biological activity. As stated in the previous Office Action and below, successful practice of the claimed invention involves the production of low antigenicity, low immunogenicity, and active factor VIII molecules and the specification does not provide guidance as to what residues, other than residue 486 of the A2 epitope, may be changed without eliminating the biological activity. The specification suggests modifying glutamine 2189 to asparagine but does not indicate whether such a factor VIII mutant would maintain activity. Given the state of the prior

art as discussed in the previous Office Action and below, it appears highly unpredictable as to what effect *amino acid changes* and *inserted glycosylation sites* would have on the biological activity of Factor VIII.

5. Applicants state that Aly et al. indicate that the loss of protein function as a result of abnormal additional glycosylation is a rare cause of clinical disorder (see Aly et al. p. 4936, last paragraph in left column). This is a general statement and implies that amino acid mutations that cause additional glycosylation in nature have rarely been reported. This statement does not indicate whether or not mutations causing additional glycosylation or additional glycosylation itself affects protein activity. Aly et al. goes on to state that mutations that cause additional glycosylation have been found in blood proteins such as anti-thrombin III and fibrinogen and that either the mutations or the glycosylation itself resulted in lower protein activity (see Aly et al. p. 4936, Col. 1, entire last paragraph). The examiner notes that there are two factors that may cause factor VIII inactivity due to the claimed method of its modification: 1) mutation of an undefined number of amino acids at relatively undefined positions and 2) adding additional glycosylation to the factor VIII protein. The claimed methods encompass removing at least one amino acid to an entire domain of Factor VIII and replacing it with the glycosylation sequence claimed. The Specification has not provided guidance or examples except for amino acid position 486 of SEQ ID NO:2 as to what amino acids can be replaced and where glycosylation may be added without eliminating Factor VIII function. Thus, in light of the Aly et al. reference, which teaches that both glycosylation

and amino acid modification can lead to the non-functional factor VIII, it appears that the Specification does not teach how to use the claimed method in its entire scope.

6. Applicants also argue that the role of the carbohydrate in FVIII function is dispensable. Applicants refer to the Aly et al. showing that neuramidase, β-Gal, and N-glycanase did not inactivate FVIII and conclude that FVIII having a modified glycosylation would be biologically active. This argument has been considered but is not deemed persuasive because 1) the removal of glycosylation using neuramidase, β-Gal, and N-glycanase does not require mutation of the FVIII sequence as required by the claim and 2) Aly et al. indicate that introduction of glycosylation sites into FVIII (as is done in the method presently claimed) inactivates the protein. Thus, the rejection is maintained.

7. At present, the claims are broadly drawn to a method of making a mutation anywhere in the A2 or C2 domains to introduce a glycosylation site. Thus, the claims encompass making at least one amino acid substitution and can include substituting any number of amino acid residues with the claimed glycosylation sequence.

Production of inhibitory antibodies that inactivate factor VIII is a problem in the art of treating hemophilia A by administering factor VIII. A review of the specification appears to indicate that the utility of the claimed method lies in the product that is made; a functional factor VIII molecule that evades detection by inhibitory antibodies. The invention addresses solving this problem of factor VIII inactivation by producing factor VIII molecules with glycosylation sites inserted into the epitopes recognized by these inhibitory antibodies in order to shield the epitopes from recognition. Thus, successful

practice of the claimed method involves the production of low antigenicity, low immunogenicity, and active factor VIII molecules.

8. However, Aly et al. (Proc. Natl. Acad. Sci USA, June 1992, Vol. 89, pp. 4933-4937) indicate that the introduction of glycosylation sites at certain positions of the factor VIII molecule inactivate the protein. Aly et al. teach the identification of two hemophilia patients with non-functional factor VIII proteins wherein abnormal glycosylation in the light chain and in the A2 domain blocks the factor VIII procoagulant activity (see abstract, and Discussion, p. 4936). It appears that at the time of the invention, it was surprising to find that glycosylation could affect protein function of factor VIII. Aly et al. do not propose how the glycosylation affected the procoagulant activity and the present specification nor any other art reference at the time of the invention does not supplement this information. Thus, since one of skill in the art did not understand how glycosylation affects procoagulant activity, it would have been impossible to predict what affect a glycosylation site at a given amino acid position would have.

9. In addition to the complexity created by the lack of understanding of how additional glycosylation affects factor VIII activity, the prior art acknowledges, as evidence by Aly et al., the difficulty of understanding the effects of any particular point mutations in the factor VIII molecule due to its large size and many exons (p. 4933, Col. 1, 2nd paragraph). And, the effect of any amino acid modifications in the factor VIII sequence is unpredictable given, not only the complexity of its structure, but also its activity. Factor VIII participates in blood coagulation as an essential cofactor in the cleavage of factor X by factor IXa in the presence of Ca^{++} and phospholipid. Factor VIII

is produced as a single-chain protein of 2351 amino acids and is modified by proteolytic cleavages to generate amino terminal heavy chain polypeptides and a carboxy-terminal light chain. Procoagulant activity further requires thrombin cleavage of the factor VIII heavy and light chains to form a heterotrimer of subunits A1 and A1 from the heavy chain and subunit A3-C1-C2 from the light chain. Therefore, the art acknowledged unpredictability of amino acid modification on protein function especially applies to a complex molecule such as factor VIII given that the modification could affect any one of the events required for procoagulant activity. Such required events could include, for example, inhibition of one of the proteolytic cleavages due to disruption of cleavage site or disruption of protease binding site, structural changes that prevent heterotrimer formation, or structural changes that impair formation of the enzymatic complex.

10. Despite the unpredictability of the effect of amino acid modification on factor VIII function, the specification only provides an example of successfully using the method to produce active factor VIII molecules with a specific additional glycosylation site (leucine 486 of SEQ ID NO:2 is substituted with asparagine (L486N)). The specification also suggests introducing a glycosylation site in the light chain by replacing glutamine 2189 with asparagine (Q2189N)) but does not address whether it retains its procoagulant activity. Thus, while the consensus sequence for glycosylation and recombinant means for making mutations in proteins were very well established at the time of the invention, it was not routine in the art to screen for positions within a protein's sequence where amino acid modifications (in this case both amino acid change and addition of glycosylation) can be tolerated. Obtaining both the desired functionality and structure

(in this case glycosylation) of the factor VIII protein requires knowledge of and guidance as to what amino acids in the sequence are tolerant to modification and a detailed knowledge of the ways in which the factor VIII structure relates to its function (what affect does glycosylation have on activity to allow one to successfully predict what parts of the protein structure could be glycosylated while maintaining activity). Such experimentation is undue.

11. Due to the large quantity of experimentation necessary to determine what amino acid positions in the factor VIII sequence could be modified to insert a glycosylation site that would result in an active factor VIII protein; the lack of direction/guidance presented in the specification regarding how glycosylation affects factor VIII procoagulant activity; the absence of working examples for methods of preparing a factor VIII molecule having glycosylation sites at positions other than 486; the complex nature of the invention; the state of the prior art which establishes the unpredictability of the effects of mutation on protein structure and function and establishes that abnormal glycosylation blocks factor VIII procoagulant activity for undetermined reasons; and the breadth of the claims which fail to recite any structural limitations as to the position of the desired mutation, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope. To practice the instant invention in a manner consistent with the breadth of the claims would not require just a repetition of the work that is described in the instant application but a substantial inventive contribution on the part of a practitioner involving the determination of those amino acid residues in a factor VIII molecule that can be modified to successfully produce an active factor VIII with

additional glycosylation. It is this additional characterization of the protein that is required in order to obtain the functional and structural data needed to permit one to produce a protein which meets both the structural and functional requirements of the instant claims that constitutes undue experimentation.

12. The examiner notes that claims 4 and 5 are still drawn to making mutations anywhere in the FVIII sequence because the claim is only limited to mutating a segment that comprises the amino acid residue, leucine, at position3 of SEQ ID NO:2. An example of an amendment that would overcome the rejection was provided in the previous Office Action (p. 4 of Paper No. 12) and repeated on pages 9-10 of this Office Action.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 is indefinite because the claim recites an amino acid position without reference to a sequence to place the position in the context of a full-length protein. A protein, even from a single species, rarely has a single sequence or length in all members of the family or species. Mutations causing disease or merely allelic

variations provide various different sequences for a single protein. Thus, the position of 2189 in the C2 domain of human factor VIII is indefinite.

Applicants have not addressed this rejection and the amendment to Claim 5 does not overcome the rejection. Therefore, the rejection is maintained.

Conclusions

Claim 6 appears to be in condition for allowance. A thorough search of the sequence database and the prior art did not reveal any factor VIII sequences wherein the leucine at residue 3 of SEQ ID NO:2 of the A2 domain was replaced with asparagine. Furthermore, there was no teaching or suggestion in the prior art of record of making such a modified factor VIII protein.

Claims 1 and 5 are rejected for the reasons cited above.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Holly Schnizer whose telephone number is (703) 305-3722. The examiner can normally be reached on Tuesday, Thursday, and Friday from 8 am to 5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on (703) 308-2923. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703 308-0196.

Holly Schnizer
October 21, 2003

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